PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	10 FEB 2009
Applicant's or agent's file reference 78341-A-PCT/JPW/DZ	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 08/11891	17 October 2008 (17.10.2008)	19 October 2007 (19.10.2007)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C12Q 1/68 (2009.01)
USPC - 435/6

Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	27 January 2009 (27.01.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 08/11891

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed

      ☐ filed together with the international application in electronic form

      ☐ furnished subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 08/11891

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-76	YES
	Claims	none	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-76	NO
Industrial applicability (IA)	Claims	1-76	YES
	Claims	none	NO

2. Citations and explanations:

Claims 1-9, 11-12, 17-19 and 21-24 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0185466 A1 to JU et al. (hereinafter 'Ju 466').in view of US 2006/0160081 A1 to MILTON et al. (hereinafter 'Milton')

As per claim 1, Ju '466 discloses a composition having a first, second and third portion wherein the wherein A represents a point of attachment to the first portion and B represents a point of attachment to the third portion (para [0123]). Ju '466 does not disclose a second portion having the structure as claimed. Milton further discloses azide groups located adjacent to substituted aryl groups (para [0032]), useful for cleavage of a linker connecting a detectable label to a nucleotide analog (para [0025]). It would have been obvious to modify the linking group taught by Ju '466 (para [0123]) to a phosphine-cleavable linker as taught by Milton to obtain the invention as claimed, because it provides convenient chemical cleavage of a label as taught by Milton (para [0025]).

As per claim 2, Ju '466 further discloses wherein the first portion comprises a deoxynucleotide or a dideoxynucleotide and the third portion comprises a detectable marker (para [0123]).

As per claim 3, Ju '466 further discloses wherein the detectable marker is a fluorescent dye (para [0126]).

As per claim 4, Ju '466 discloses the composition of claim 2, wherein the first portion is a deoxynucleotide (para [0123]) but does not disclose a nucleotide comprising a methylazido group attached to a 3' 0 atom thereof. Milton discloses methylazido groups useful for cleavably labelling nucleotides (para [0012]-[0018]). It would have been obvious to use the cleavable methylazido group as taught by Milton, in the cleavable 3' O position of a nucleotide analog, as taught by Ju '466 (para [0137]), to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claim 5, Ju '466 discloses the composition of claim 3, wherein the dye in each structure is a fluorescent dye (para [0126]), but does not disclose wherein the structure comprises a 3' O methylazido group. Milton further discloses methylazido groups (para [0012]-[0018]) and azide groups (para [0029]-[0032]) useful for cleavably labelling nucleotides. It would have been obvious to use the cleavable methylazido and azide group as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claim 6, Ju '466 (para [0126]) and Milton (para [0012]-[0018], [0029]-[0032]) suggest the composition of claim 5 having the structure as claimed. Ju '466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094], [0095]), but does not disclose clevage via azide groups. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466 and Milton by one of ordinary skill in the art without undue experimentation.

As per claims 7-8, Ju '466 discloses a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid (para [0007], [0008]), or in a self priming nucleic acid (para [0087]) comprising:
a) contacting the nucleic acid with (i) at least four different deoxynucleotide triphosphate (dNTP) analogues, each having the structure as claimed: (para [0126]) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine, wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, and wherein L is a cleavable linker molecule comprising the structure: (para [0126]) wherein A represents a point of attachment to the base and B represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group, (para [0126]) (ii) a nucleic acid polymerase and (iii) a nucleic acid primer which hybridizes with the nucleic acid, under conditions permitting one of the four dNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of the nucleic acid primer and thereby extend the primer (para [0007]-[0008], [0065]-[0077]);

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Box No. V. 2. Citations and explanations:

(claims 7-8 cont'd) b) identifying the fluorophore of the dNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; (para [0065]-[0077]);
d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified (para [0065]-[0077]);
e) repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid.(para [0065]-[0077]). Ju '466 does not disclose c) contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP. Milton further discloses methylazido groups (para [0012]-[0018]) and azide groups (para [0029]-[0032]) useful for cleavably labelling nucleotides. It would have been obvious to use the cleavable methylazido and azide group as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claim 9, Ju '466 further discloses wherein steps b) and c) can be performed simultaneously, or in the order step b) then step c) or in the order step c) then step b) (para [0075]).

As per claim 11, Milton further discloses wherein the cleavable chemical group is a methylazido group (para [0029]-[0032]).

As per claim 12, Ju '466 and Milton suggest the method of claim 7 or 8, wherein the four dNTP analogues have the structures as claimed. Ju '466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094]-[0095]), but does not disclose clevage via azide groups. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466 and Milton by one of ordinary skill in the art without undue experimentation.

As per claim 17, Ju '466 further discloses wherein the nucleic acid is immobilized on a solid surface (para [0010]).

As per claim 18, Ju '466 further discloses wherein the solid surface is a chip or a bead (para [0010]).

As per claim 19, Ju '466 does not explicitly disclose a kit for use in sequencing a nucleic acid comprising:
(a) a plurality of four nucleotide analogues having the the structures as claimed, and instructions for use. Ju466does disclose a set of four bi-cleavable fluorescently labelled deoxynucleotide analogs,and dideoxy nucleotide analogs (para [0032]) but does not disclose the cleavable azide groups. Milton further discloses wherein the cleavable chemical groups are methylazido and azide groups (para [0029]-[0032]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466 and Milton by one of ordinary skill in the art without undue experimentation. It would have been obvious to assemble the nucleotide analogs suggested by Ju '466and Milton into a kit together with instructions for use by others, to obtain the invention as claimed, because it would provide a means for one of ordinary skill in the art to implement the disclosed methods, and kits with instructions were well known in the art at the time the invention was made.

As per claims 21-22, Ju '466 discloses an array comprising a self-priming nucleic acid attached to a solid surface (para [0138]), but does not disclose wherein the nucleic acid comprises an azidomethyl group attached to a 3' 0 atom thereof and a molecule having the structure as claimed, wherein A represents a point of attachment to a 3' base of the nucleic acid and B represents a point of attachment to a detectable marker. Ju '466 also discloses cleavable fluorescent labels and 3' O blocking groups (para [0094]-[0095]), but not the azidomethyl group. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031]-[0033]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466 and Milton by one of ordinary skill in the art without undue experimentation.

As per claim 23, Ju '466 further discloses wherein the detectable marker is a fluorophore (para [0126]).

As per claim 24, Ju '466 discloses a method for increasing a read length of DNA sequencing by synthesis comprising (a) providing deoxynucleotide triphosphate analogues wherein the deoxynucleotide triphosphate analogues differ from deoxynucleotide triphosphates by having a cleavable group attached to a 3' 0 atom (para [0137]) thereof and by having a detectable marker attached to a 1 nitrogen or a 9 nitrogen of a base thereof (para [0126]-[0131]) wherein A represents a point of attachment to a the base and B represents a point of attachment to the detectable marker (para [0126]-[0131]); and (b) incorporating a plurality of the deoxynucleotide triphosphate analogues into a nucleic acid being synthesized in the DNA sequencing by synthesis and (c) cleaving the cleavable group (para [0137]). Ju '466 does not precisely disclose a linker comprising the structure as claimed, or a cleavable methylazido group. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031]-[0033]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466 and Milton by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide group as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

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Claims 10, 13-16 and 20 lack an inventive step under PCT Article 33(3) as being obvious over Ju '466 in view of Milton and further in view of the article entitled "Four-color DNA sequencing by synthesis using cleavable fluorescent nucleotide reversible terminators" by JU et al. (hereinafter "Ju-PNAS").

As per claim 10, Ju '466 and Milton, suggest the method of claim 7 or 8, wherein the nucleic acid is DNA (Ju '466, para [0007]-[0008]), but do not disclose wherein the nucleic acid polymerase is a 9N thermopolymerase. Ju-PNAS discloses a method of nucleic acid sequencing (pg 19635, abstract) wherein 9 N thermopolymerase is employed to incorporate cleavable nucleotide analogs into an elongating primer product (pg 19640, materials and methods, para 4). It would have been obvious to use 9 N polymerase as taught by Ju-PNAS in the sequencing methods suggested by Ju '466 and Milton, to obtain the invention as claimed, because it was a polymerase known to effectively incorporate nucleotide analogs.

As per claims 13 and 20, Ju '466 and Milton suggest the method of claim 12, 30, and the kit of claim 19, but do not explicitly disclose wherein the four dNTP analogues or the four ddNTP analogs have the structures further comprising the fluorescent moieties as claimed. JuPNAS discloses cleavable nucleotide analogs further comprising the fluorescent moieties as claimed (pg 19636 Fig. 1). It would have been obvious to use the flourescent moieties taught by JuPNAS, in the cleavable nucleotide analogs suggested by Ju466and Milton, to obtain the invention as claimed, because they were well known fluorophores commonly employed in fluorescent DNA sequencing methods.

As per claims 14-16, Ju '466 and Milton suggest the method of claim 7 or 8, but do not disclose wherein up to 1000, 10,000, or 1,000,000 consecutive nucleotides are identified. Ju-PNAS does not explicitly disclose wherein up to 1,000,000 consecutive nucleotides are identified using a single primer extension, but does anticipate optimization of sequence by synthesis methods, such as those claimed, wherein throughput will generate over 20 million base-reads per chip (pg 19640 para 1). One of ordinary skill in the art would recognize that by properly choosing multiple primer sites, and optimizing nucleotide and nucleotide analog mixes, reads of up to 1,000,000 consecutive nucleotides would be achievable.

Claims 25-27, 29, 30, 32-34, 36-41, 46-48 and 50-76 lack an inventive step under PCT Article 33(3) as being obvious over Ju '466 in view of Milton and further in view of US 2005/0170367 A1 to QUAKE et al. (hereinafter 'Quake').

As per claims 25-26 and 32-33, Ju '466 discloses a method for determining the identity of each of a series of consecutive nucleotide residues in a self priming nucleic acid (para [0007]-[0008], [0087]), or a plurality of self-priming nucleic acids (Fig. 2) comprising:
a. contacting the nucleic acid with (i) at least four different deoxynucleotide triphosphate (dNTP) analogues, each having the structure as claimed: (para [0126]) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine, wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, and wherein L is a cleavable linker molecule similar to the structure as claimed: (para [0126]), wherein A represents a point of attachment to the base and B represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group, (ii) a nucleic acid polymerase and (iii) a nucleic acid primer which hybridizes with the nucleic acid, under conditions permitting one of the four dNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of the nucleic acid primer and thereby extend the primer (para [0007]-[0008], [0065]-[0077]);
b. identifying the fluorophore of the dNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide (para [0065]-[0077]);
d. iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified (para [0065]-[0077]);
e. repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid (para [0065]-[0077]). Ju '466 does not disclose c. contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c. contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP (para [0044]). Milton further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. In addition, Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake, to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

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As per claims 27 and 34 Ju '466 further discloses wherein steps b) and c)can be performed simultaneously, or in the order step b) then step c) or in the order step c) then step b) (para [0075]).

As per claim 29 and 36, Milton further discloses wherein the cleavable chemical group is a methylazido group (para [0080]).

As per claim 30, Ju '466 (para [0126], Milton(para [0031], [0033], [0080), and Quake (para [0010]-[0012], [0055]) suggest wherein the four dNTP analogues have the structures as claimed. Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466 or Milton, these compounds could have been derived from the teachings of Ju '466, Milton, and Quake by one of ordinary skill in the art without undue experimentation.

As per claim 37, Milton further discloses wherein the linker on the ddNTP and the chemical group on the 3' 0 position of the dNTP is cleaved with a phosphine (para [0080]-[0081]).

As per claim 38, Milton further discloses wherein the phosphine is charged and water soluble (para [0044]).

As per claim 39, Milton further discloses wherein the phosphine is tris (2-carboxyethyl) phosphine (para [0044]).

As per claims 40-41, Ju '466, Milton, and Quake suggest wherein the four ddNTP analogues have the structures as claimed. Ju466teaches nucleotide analogs with cleavable fluorescent tags and cleavable 3' O blocking groups (para [0007], [0126]), and Milton teaches use of an azide group to provide specific cleavage at predetermined sites (para [0080]-[0081]). Milton further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466or Milton, these compounds could have been derived from the teachings of Ju '466and Milton by one of ordinary skill in the art without undue experimentation.

As per claim 46, Ju '466 further discloses wherein the nucleic acid is immobilized on a solid surface (para [0010]).

As per claim 47, Ju '466 further discloses wherein the solid surface is a chip or a bead (para [0010]).

As per claim 48, Ju '466 does not explicitly disclose a kit for use in sequencing a nucleic acid comprising:
(a) a plurality of four nucleotide analogues having the the structures as claimed, and instructions for use. Ju '466does disclose a set of four bi-cleavable fluorescently labelled deoxynucleotide analogs,and dideoxy nucleotide analogs (para [0032]) but does not disclose the cleavable azide groups. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c. contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP; (para [0044]). Milton also further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides. It would have been obvious to assemble the nucleotide analogs suggested by Ju '466, Milton, and Quake into a kit together with instructions for use by others, to obtain the invention as claimed, because it would provide a means for one of ordinary skill in the art to implement the disclosed methods.

As per claims 50-51, Ju '466 discloses an array comprising a self-priming nucleic acid attached to a solid surface (para [0138]), but does not disclose wherein the nucleic acid comprises an azidomethyl group attached to a 3' 0 atom thereof and a molecule having the structure as claimed, wherein A represents a point of attachment to a 3' base of the nucleic acid and B represents a point of attachment to a detectable marker. Ju ' 466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094], [0095]), but not the azidomethyl group. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c. contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP; (para [0044]). Milton further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides

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As per claim 52, Ju '466 further discloses wherein the detectable marker is a fluorophore (para [0126]).

As per claim 53, Ju '466 discloses a method for increasing a read length of DNA sequencing by synthesis coupled with Sanger dideoxynucleotide terminating reaction (para [0032])
(a) providing deoxynucleotide triphosphate analogues wherein the deoxynucleotide triphosphate analogues differ from deoxynucleotide triphosphates by having a cleavable group attached to a 3' 0 atom thereof and providing dideoxynucleotide triphosphate analogues wherein the dideoxynucleotide triphosphate analogues differ from dideoxynucleotide triphosphates by having a detectable marker attached to a 1 nitrogen or a 9 nitrogen of a base thereof through a linker (para [0126]), wherein A represents a point of attachment to a the base and B represents a point of attachment to the detectable marker (para [0126]);
(b) incorporating a plurality ratio of dideoxynucleotide triphosphate to deoxynucleotide triphosphate analogues into a nucleic acid being synthesized in the DNA sequencing by synthesis and (para [0032]);
(c) cleaving the detectable marker from each incorporated dNTP analogue, so as to thereby increase the read length of the DNA sequence by synthesis (para [0032]). Ju '466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094]-[0095]), but not the azidomethyl group. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c). contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP (para [0044]). Milton also further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claim 54, Ju '466 discloses a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: the steps a) through e), and h) through n) (para [0007]-[0008], [0036], [0065]-[0077], [0126]). Ju '466does not explicitly disclose f) denaturing the extended primer so as to de-hybridize it from the nucleic acid; or g) contacting the nucleic acid wi th (iii) a second nucleic acid primer which hybridizes with the nucleic acid. However, Ju '466does disclose attachment of a primer to a single-stranded (or de-hybridized) template in order to further extend the primer in a sequencing reaction (para [0040], Fig. 6). It would have been obvious to one of ordinary skill in the art that a second primer could be attached, as taught by Ju '466, to extend the sequencing reaction.
Ju '466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094]-[0095]), but does not disclose cleavage via azide groups. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c). contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP (para [0044]). Milton also further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claim 55, Ju '466 and Milton suggest the method of claim 54, but do not precisely disclose wherein the linker in each of step a) and j) independently each comprise the structures as claimed, wherein A represents a point of attachment to the base and B represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group. Ju '466discloses cleavable fluorescent labels and 3' O blocking groups (para [0094]-[0095]), but does not disclose cleavage via azide groups. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c). contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP; (para [0044]). Milton also further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

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Supplemental Box

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As per claim 56, Milton further discloses wherein a linker is cleaved by contacting the linker with tris(2- carboxyethyl) phosphine (para [0044]).

As per claim 57, Ju '466 further discloses wherein one or more linkers are photocleavable or chemically cleavable (para [0007], [0008]).

As per claim 58, Ju '466 further discloses wherein one or more chemical groups are photocleavable or chemically cleavable (para [0007], [0008]).

As per claim 59, Milton further discloses wherein R in the structures set forth in steps a) and or j) is independently chosen from a -N3 group (para [0033]) or an allyl group (para [0037]).

As per claim 60, Milton further discloses wherein the cleavable chemical group in step g) is independently chosen from the a -N3 group (para [0033]) or an allyl group (para [0037]).

As per claims 61 and 69, Ju '466, Milton, and Quake suggest a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising steps a). through f),:and j). through n), further comprising a second primer as in step g) (iii) or (iv) (see explanation for claim 54 above). Ju '466, Milton, or Quake do not disclose use of only three different types of deoxynucleotide triphosphates, or different types of deoxynucleotide triphosphates combined with a deoxynucleotide triphosphate analogue, differing from a deoxynucleotide triphosphate by having a cleavable chemical group attached to the 3' O-atom of the dNTP analogue and differing from the three different types of deoxynucleotide triphosphates by having a different base therefrom. However, manipulation of nucleotide mixes in order to selectively fill in nucleotide gaps or terminate primer extensions, or manipulate the length of an extension before termination, etc. were well known in the art. A proper nucleotide mix to achieve the desired fill-in or primer extension effect would have been obvious to one of ordinary skill in the art without undue experimentation

As per claims 62 and 70, Ju '466 further discloses wherein in steps g) and h) the three types of dNTPs are chosen from the group dATP, dCTP, dGTP, dTTP or dITP (para [0036]).

As per claims 63 and 71, Ju '466 and Milton suggest the method of claim 61, wherein the linker in each of step a)and j) independently each comprise the structures as claimed wherein A represents a point of attachment to the base and B represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group. Ju '466 discloses cleavable fluorescent labels and 3' O blocking groups (para [0094], [0095]), but not the azidomethyl group. Milton further discloses use of an azide group to facilitate cleavage of nucleotide analogs at predetermined positions (para [0031], [0033]), and c). contacting the dNTP analogue which has formed the phosphodiester bond with tris(2-carboxyethyl) phosphine so as to thereby (1) cleave the fluorophore and (2) cleave the cleavable chemical group from the dNTP; (para [0044]). Milton also further discloses a phosphine-cleavable linker wherein the azide group is a substituent of a glycol portion of the linker (para [0128]), as in the linker as claimed. Quake further discloses extended chain cleavable linkers (para [0010]-[0012]) for fluorescently tagged nucleotides, wherein the linker comprises glycol moieties for improved solubility (para [0055]). Although the precise arrangement of atoms in the cleavable nucleotides as claimed are not explicitly disclosed by Ju '466, Milton, or Quake, these compounds could have been derived from these teachings of by one of ordinary skill in the art without undue experimentation. It would have been obvious to use the cleavable methylazido and azide groups, and carboxy ethyl phosphine cleavage reagent, as taught by Milton, in the cleavable 3' O position of a nucleotide analog, and the cleavable linker, as taught by Ju '466 (para [0137]), or Quake to obtain the invention as claimed, because it provides an efficient means for reversibly terminating nucleic acid chain elongation and detecting incorporated nucleotides.

As per claims 64 and 72, Milton further discloses wherein a linker is cleaved by contacting the linker with tris (2-carboxyethyl) phosphine (para [0044]).

As per claims 65 and 73, Ju '466 further discloses wherein one or more linkers are photocleavable or chemically cleavable (para [0007]-[0008]).

As per claims 66 and 74, Ju ' 466 further discloses wherein one or more chemical groups are photocleavable or chemically cleavable (para [0007]-[0008]).

As per claims 67 and 75, Milton further discloses wherein R in the structures set forth in steps a) and or j) is independently chosen from a -N3 group (para [0033]) or an allyl group (para [0037]).

As per claims 68 and 76, Milton further discloses wherein the cleavable chemical group in step g) is independently chosen from a -N3 group (para [0033]) or an allyl group (para [0037]).

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 08/11891

Supplemental Box

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Claims 28, 31, 35, 42-45 and 49 lack an inventive step under PCT Article 33(3) as being obvious over Ju '466 in view of Milton in further view of Quake and further in view of Ju-PNAS.

As per claims 28, and 35, Ju '466, Milton, and Quake suggest the method of claim 25 or 26, 32 or 33, wherein the nucleic acid is DNA (Ju '466, para [0007]-[0008]), but do not disclose wherein the nucleic acid polymerase is a 9N thermopolymerase. Ju-PNAS further discloses a method of nucleic acid sequencing (pg 19635, abstract) wherein 9 N thermopolymerase is employed to incorporate cleavable nucleotide analogs into an elongating primer product (pg 19640, materials and methods, para 4). It would have been obvious to use 9 N polymerase as taught by Ju-PNAS in the sequencing methods suggested by Ju '466 and Milton, to obtain the invention as claimed, because it was a polymerase known to effectively incorporate nucleotide analogs.

As per claims 31, 42 and 49, Ju '466, Milton, and Quake suggest the method of claim 30, 40, and the kit of claims 48, but do not explicitly disclose wherein the four dNTP analogues or the four ddNTP analogs have the structures further comprising the fluorescent moieties as claimed. Ju-PNAS further discloses cleavable nucleotide analogs further comprising the fluorescent moieties as claimed (pg 19636, Fig. 1). It would have been obvious to use the flourescent moieties taught by Ju-PNAS, in the cleavable nucleotide analogs suggested by Ju466and Milton, to obtain the invention as claimed, because they were well known fluorophores commonly employed in fluorescent DNA sequencing methods.

As per claims 43-45, Ju '466, Milton, and Quake suggest the method of claim 7 or 8, but do not disclose wherein up to 1000, 10,000, or 1,000,000 consecutive nucleotides are identified. While Ju-PNAS does not explicitly disclose wherein up to 1,000,000 consecutive nucleotides are identified using a single primer extension, Ju-PNAS does suggest optimization of sequence by synthesis methods, such as those claimed, wherein throughput will generate over 20 million base-reads per chip (pg 19640, para 1). One of ordinary skill in the art would recognize that by properly choosing multiple primer sites, and optimizing nucleotide and nucleotide analog mixes, reads of up to 1,000,000 consecutive nucleotides would be achievable.

Claims 1-76 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.